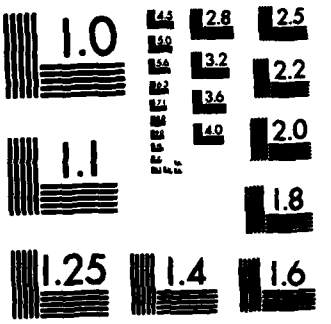
AD-A143 957 MULTINUCLEATED GIANT CELLS INDUCED FROM MONOCYTE PRECURSORS BY MONOCLONAL.. (U) JOHNS HOPKINS UNIV BALTIMORE MD DEPT OF PHARMACOLOGY J E HILDRETH ET AL. 1/1

UNCLASSIFIED 02 AUG 84 N00014-82-K-0221 F/G 6/5 NL

END




MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

(2)

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

| REPORT DOCUMENTATION PAGE | | READ INSTRUCTIONS BEFORE COMPLETING FORM |
|---|---|---|
| 1. REPORT NUMBER | 2. GOVT ACCESSION NO. | 3. RECIPIENT'S CATALOG NUMBER |
| 4. TITLE (and Subtitle) Multinucleated giant cells induced from monocyte precursors by monoclonal anti-HLA-DR antibodies | | 5. TYPE OF REPORT & PERIOD COVERED Technical report<br>6. PERFORMING ORG. REPORT NUMBER |
| 7. AUTHOR(s) James E. K. Hildreth and J. Thomas August | | 8. CONTRACT OR GRANT NUMBER(s) N00014-82-K-0221 |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS Johns Hopkins University School of Medicine Department of Pharmacology, 725 N. Wolfe Street Baltimore, MD 21205 | | 10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NR 202-231 |
| 11. CONTROLLING OFFICE NAME AND ADDRESS | | 12. REPORT DATE 8/2/84<br>13. NUMBER OF PAGES |
| 14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) | | 15. SECURITY CLASS. (of this report)<br>15a. DECLASSIFICATION/DOWNGRADING SCHEDULE |

16. DISTRIBUTION STATEMENT (of this Report)

Distribution unlimited

DISTRIBUTION STATEMENT A
Approved for public release
Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

DTIC ELECTE AUG 9 1984 S D B

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-LF-014-6601

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

84 08 09 007

AD-A143 957

DTIC FILE COPY

(2)

# Multinucleated giant cells induced from monocyte precursors by monoclonal anti-HLA-DR antibodies

James E. K. Hildreth and J. Thomas August

Department of Pharmacology and Experimental Theraputics

The Johns Hopkins School of Medicine

Multinucleated giant cells (MGC) have been observed in inflamed tissues for over a century (1). MGC form in granulomas caused by bacterial and viral infections and granulomas induced by physical or chemical agents (1). MGC are also encountered in granulomatous diseases of unknown etiology such as sarcoidosis, rheumatoid arthritis, and Crohn's disease (1). Recent studies have shown that MGC are the result of macrophage fusion (2-5). It has been suggested that fusion results when macrophages are overwhelmed with antigen, such as in a granulomatous environment, and several macrophages simultaneously attempt to phagocytose the antigen (6).

In addition to the phagocytosis of foreign antigen, macrophages also present antigen in association with products of class II major histocompatibility complex (MHC) genes to antigen-specific T cells (7). The association of foreign antigen with class II MHC products (Ia antigens) suggests that Ia antigens may be involved in foreign antigen-induced formation of MGC. We report here that monoclonal antibodies against human Ia antigens (HLA-DR) induce rapid formation of MGC from monocyte precursors.

Two hours after adding HLA-DR specific MAb to human monocyte cultures large aggregates of adherent and non-adherent cells were observed (Figure 1a). These aggregates, which were not observed in cultures without antibody, accounted for most of the cells. After 24 hours of culture with HLA-DR specific MAb almost all of the cells were gathered into very large clusters consisting of several cells (Figure 1b). In control cultures a uniform monolayer of adherent cells was observed at this time. After an additional 24 hours of culture many of the large clusters had differentiated into MGC of the foreign-body type (FB-MGC) (Figure 1c). In addition, the predominant cell type at day 2 was a very large multinucleated (10-60 nuclei) polygonal epitheloid cell with 2 to 4 cytoplasmic extensions. Six to 12 hours later these cytoplasmic extensions had spread and fused, resulting in very large

FB-MGC. After 72 hours of culture with the HLA-DR specific MAb, 80-90 percent of the nuclei were seen in MGC (Fig. 1d). The MGC continued to fuse such that by 4 to 5 days of culture, polykaryons with greater than 100 nuclei could be observed. In monocyte cultures without HLA-DR specific MAb, greater than 90 percent of the cells were present in the non-adherent population after 3 days of culture, as previously reported (8). The adherent cells in control cultures showed no signs of fusion. The anti-HLA-DR induced MGC could be cultured for up to 30 days and maintained the morphology of foreign-body type MGC (Figure 2) with the nuclei found randomly organized in the cytoplasm. MGC of the Langhans type with the nuclei forming a ring around the cell center were occasionally seen after extended culture periods. Monocytes treated with anti-HLA-DR MAb did not incorporate $^{3}$H-thymidine confirming that MAb-induced MGC formed by fusion not by nuclear division (data not shown).

MGC were induced by MAb against HLA-DR antigens but not by MAb against other antigens present on monocytes (Table 1). Aggregation and fusion of monocytes to form MGC could be induced by HLA-DR specific MAb of three different isotypes. A MAb (MHM 5) against class I MHC antigens (HLA-A,B,C) induced aggregation of monocytes but not fusion. Similarly, a MAb (H5A4) specific for the human monocyte-macrophage differentiation antigen HMac-1 (9) induced cell aggregates but these did not fuse to form MGC. MAb against two other antigens expressed on monocytes, the leukocyte common antigen (H5A5) and a monocyte specific polypeptide of Mr 200,000 (H6A7), did not induce aggregation or fusion. These results indicated that MGC formation did not result simply from MAb-induced aggregation of monocytes, but rather occured through a more complex mechanism involving Ia antigens. The results also suggested that Fc receptors were not involved in anti-HLA-DR induced MGC formation since the isotypes of the MAb which failed to induce MGC formation were the same as those of the HLA-DR specific MAb. Studies using $F(ab')_2$

fragments of an anti-HLA-DR MAb (MHM.33) confirmed that the Fc region of the immunoglobulin was not required for MGC induction (data not shown). MGC were induced more effectively, however, by intact MAb possibly due to the additional aggregation of the monocytes mediated by Fc-receptors. MGC formation was not observed when monocytes were cultured with Fab' fragments of anti-HLA-DR MAb suggesting that cross-linking of HLA-DR MAb antigens on the cell surface is required for MGC formation.

The phenotype of anti-HLA-DR induced MGC was examined and compared to that of dendritic cells and activated macrophages (Table 2). The MGC did not express surface immunoglobulin but did express class I and class II MHC antigens and the human Mac-1 antigen (HMac-1). They were also positive in assays for Fc-receptor activity and showed a high degreee of antibody-dependent phagocytic activity (Table 2). This is in contrast to a previous report which showed that in vitro generated MGC were only poorly phagocytic (10). The MAb-induced MGC strongly adhered to both glass and plastic culture vessels. These results showed that the MGC had characteristics identical to those of mature activated macrophages and that the MGC were not related to dendritic cells which also express class II MHC antigens and form spreading cytoplasmic processes (11).

We found that HLA-DR MAb-induced formation of MGC required a minimum cell density of $2.5 \times 10^5$ per ml and the presence of serum. The formation of MGC occurred more readily in fetal bovine serum (FBS) than in pooled human AB serum. MGC formed in response to HLA-DR MAb in FBS containing medium in as little as 2 days whereas in medium containing human serum MGC were less frequent and were not seen before 4 days of culture. It was not determined in the present study whether the superiority of FBS resulted from nutritive, antigenic, or hormonal factors. Monocytes isolated from different normal individuals varied in their capacity to form MGC. This variability probably

reflects the degree of activation of the monocytes since the addition of crude macrophage activation factor (mixed lymphocyte culture supernatant) to the monocyte - anti-HLA-DR MAb cultures resulted in a high degree of fusion of cells from 9 different individuals (data not shown). We are currently attempting to define the nature of the factor(s) providing the second signal for MGC formation.

These results show that human Ia antigens (HLA-DR) are involved in the formation of multinucleated giant cells (MGC) from monocyte precursors. Phagocytic polykaryons formed rapidly in response to binding of anti-HLA-DR MAb to monocytes. Results of previous studies indicate that macrophages form MGC in response to the persistent presence of large quantities of antigen (6). Foreign antigens appear to associate with Ia molecules on the macrophage surface (7). Thus it is possible that Ia-specific MAb induce the formation of MGC by mimicking the effect of the association between foreign antigens and Ia molecules. Lectins (14), polyclonal antisera (15), and a lymphocyte-derived macrophage fusion factor (MFF) (2,5,12,13) have been shown to induce macrophages to form MGC in vitro. In these studies only mature alveolar or peritoneal macrophages which had been cultured 1-3 days were subject to MGC formation. This may be explained by the poor expression of Ia-antigens on resting or non-activated macrophages (11). Ia-antigens are glycoproteins which bind to commonly used lectins and thus may be involved in lectin induced MGC formation. The induction of MGC by anti-macrophage serum could be due to the presence of anti-Ia antibodies in the serum used. Our results suggest that Ia antigens may be receptors for the previously reported MFF.

Anti-Ia induced formation of MGC provides a convenient and reproducible model for studying the characteristics and functions of multinucleated giant cells.

We wish to thank Ms. Valerie Holt for expert technical assistance and Mrs. Carol McGeeney for preparation of the manuscript.

We also wish to thank Dr. Ron Schnaar for the use of the Olympus inverted microscope and camera. This research is supported in part by grants from the National Institutes of Health (P01 AI 19373-02), the Office of Naval Research (N00014-82-K-0221) and the Johnson and Johnson Focused Giving Program.

Accession For
NTIS GRA&I ☑
DTIC TAB ☐
Unannounced ☐
Justification
By
Distribution/
Availability Codes
Dist | Avail and/or Special
A-1

DTIC COPY INSPECTED

Table 1

Specificity of MAb-induced MGC

| MAb | Isotype | Specificity | Aggregation | MGC Formation |
|---|---|---|---|---|
| MHM.33[a] | IgG2a,k | HLA-DR | + | + |
| MHM.35[a] | IgM,k | HLA-DR | + | + |
| MHM.36[a] | IgG1,k | HLA-DR | + | + |
| MHM.5[a] | IgG1,k | HLA-A,B,C | + | - |
| H5A4[b] | IgG1,k | HMac-1 | + | - |
| H6A7[c] | IgM,k | p200(monocyte) | - | - |
| H5A5[c] | IgG1,k | Leukocyte common | - | - |

Table 2

Comparison of anti-HLA-DR induced MGC, dendritic cells, and macrophages

| | HLA-DR-MGC | Dendritic cell* | Macrophage* |
|---|---|---|---|
| Surface Ig | - | - | - |
| Class II MHC | + | + | + |
| Class I MHC | + | + | + |
| Mac-1 | + | - | + |
| Fc-Receptor | + | - | + |
| Glass Adherent | + | - | + |
| Phagocytic | + | - | + |

Figure 1. Time course of monoclonal antibody induced multinucleated giant cells. Peripheral blood mononuclear cells (PBMC) were isolated from healthy adult donors by Ficoll-Hypaque density centrifugation. Monocytes were isolated by centrifugation of PBMC on Percoll density gradients. Five X $10^6$ monocytes in 5 ml of RPMI-1640 (Biofluids) supplemental with 10 percent heat-inactivated fetal bovine serum (FBS) (Hyclone) were cultured in 28 $cm^2$ petri dishes (Falcon) at 37°C in a humidified atmosphere of 5 percent $CO_2$. Monoclonal anti-HLA-DR antibody (immune ascites) was added for a final dilution of 1:5000. At the indicated times, the non-adherent cells were washed away and the adherent cells were fixed with absolute methanol and stained with Giemsa. (A) 2 hours (125X); (B) 24 hours (125X); (C) 48 hours (125X); (D) 72 hours (125X).

Figure 2. Monoclonal anti-HLA-DR induced multinucleated giant cells. MGC were generated by culturing human monocytes in the presence of an HLA-DR specific MAb (MHM.33) as described in Figure 1. After 7 days of culture, cells were examined and photographed on an inverted phase microscope. The cells were then washed, fixed with absolute methanol, and stained with hematoxylin and eosin (H and E). (A) Phase contrast (100X); (B) H and E (150X).

Table 1. The specificity of MAb-induced formation of MGC. Monocytes were cultured 4 days in the presence of MAb as described in Figure 1. All MAb were used in the form of immune ascites fluid at a final dilution of 1:5000. Cultures were examined for cell aggregation and fusion every 24 hours.

[a]Magkoba et al. (16)

[b]Hildreth and August (9)

[c]Hildreth, unpublished

Table 2. Comparison of MGC, dendritic cells, and macrophages. MGC were generated by culturing monocytes with an HLA-DR specfic MAb as described in Figure 1. After 4 days of culture, non-adherent cells were removed and the MGC were then washed and fixed with absolute methanol. MAb specific for human immunoglobulin (Ig), class II MHC, class I MHC, and Mac-1 antigens were applied to the MGC and incubated for 1 hour at room temperature. After washing with PBS, goat-anti-mouse Ig peroxidase conjugate (Kirkegaard and Perry) was added and the incubation continued for 1 hour. After washing with PBS, positive staining cells were visualized by adding PBS containing diamino-benzidine (0.8 mg/ml) and $H_2O_2$ (0.03 percent). Fc-receptor and phagocytic activities were determined using antibody-coated sheep erythrocytes exactly as described (17)

*Data taken from Steinman and Nussenweig (1980) (11).

1. Papadimitriou, J.M. and Walters, M.N.-I. CRC Crit. Rev. Toxicology 6, 211-255 (1979).
2. Sone, S., Bucana, C., Hoyer, L.C., and Fidler, I.J. Am. J. Path. 103, 234-246 (1981).
3. Murch, A.R., Grounds, M.D., Marshall, C.A., and Papadimitriou, J.M. J. Path. 137, 177-180 (1982).
4. Abe, E., et al. Proc. Natl. Acad. Sci. USA 80, 5583-5587 (1983).
5. Postlethwaite, A.E., Jackson, B.K., Beachey, E.H., and Kang, A.H. J. Exp. Med. 155, 168-178 (1982).
6. Chambers, T.J. J. Path. 122, 71-80 (1977).
7. Murphy, D.B. in The Role of the Major Histocompatibility Complex in Immunobiology (ed. Darf, M.E.) 1-32 (Garland Press, New York, 1981).
8. van der Meer, J.W.M. in the Reticuloendothelial System, Vol. 1 (ed. Carr, I. and Daems, W.T.) 735-771 (Plenum Press, New York, 1980).
9. Hildreth, J.E.K. and August, J.T. Manuscript submitted for publication.
10. Papadimitriou, J.M., Robertson, T.A., and Walters, M.N.-I. Am. J. Path., 78, 343-353 (1975).
11. Steinman, R.M. and Nussenweig, M.C. Immunological Rev. 53, 127-147 (1980).
12. Warfel, A.H. and Hadden, J.W. Am. J. Path. 93, 753-766 (1978).
13. Warfel, A.H. and Hadden, J.W. Exp. Mol. Path. 33, 153-168 (1980).
14. Chambers, T.J. J. Path. 123, 53-61 (1977).
15. Chambers, T.J. J. Path. 122, 163-173 (1977).
16. Makgoba, M.W., Hildreth, J.E.K., and McMichael, A.J. Immunogenetics 17, 623-635 (1983).
17. Herscowitz, H.B., Holden, H.T., Bellanti, J.A., and Ghaffar, A. eds. Manual of Macrophage Methodology (Marcel Dekker, New York, 1981).

FIGURE 1

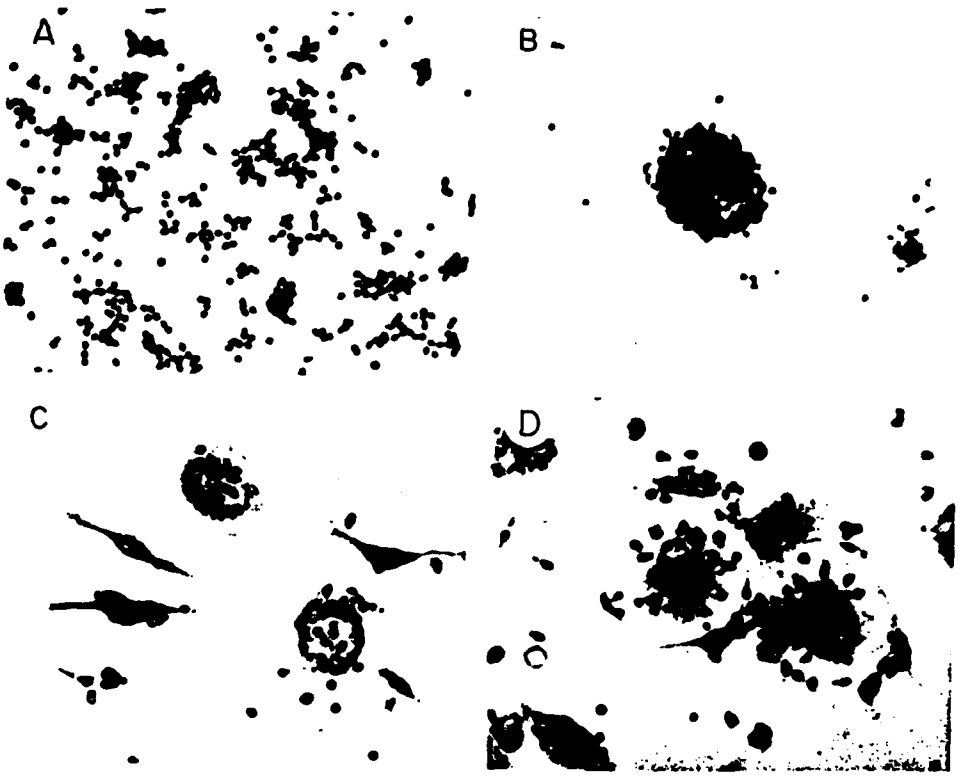
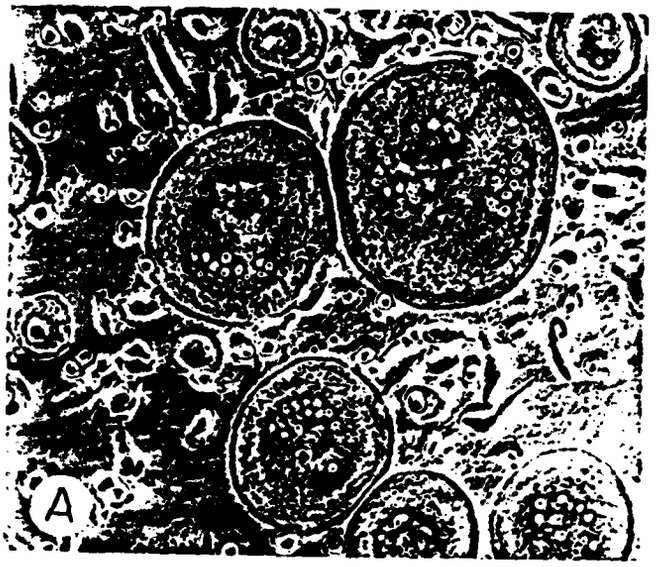
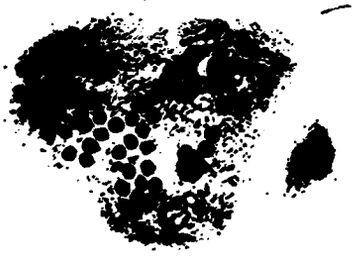
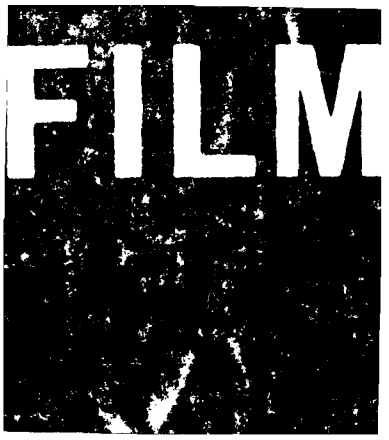


FIGURE 2



B


FILM